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Case Report: Multiple beta-catenin mutations in hepatocellular lesions arising in Abernethy Malformation

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Abstract

An 18 year old man underwent liver transplantation due to an Abernethy malformation associated with multiple hepatocellular nodules including one which was a rapidly enlarging and was suspicious for malignant transformation. Analysis of the explanted liver showed a spectrum of multiple hepatocellular nodules ranging in appearance from focal nodular hyperplasia, hepatocellular adenoma and to a well differentiated hepatocellular neoplasm borderline for hepatocellular carcinoma. Mutational analysis revealed wildtype beta-catenin expression in the background liver and some nodules, whilst different variants were present in other lesions irrespective of their morphological appearance. No telomerase reverse transcriptase (TERT) promoter mutation was identified.

Conclusion: Abernethy malformations can lead to independent genetic events which can result in beta-catenin mutations associated with malignant transformation of hepatocellular nodules. When following up such patients one must therefore have a high index of suspicion, particularly if radiological surveillance reveals a change in the nature of hepatic lesions.

Introduction

Abernethy malformation is the eponym applied to congenital extrahepatic portosystemic shunts, a rare anatomical abnormality in which the splanchnic venous flow bypasses the liver and drains directly into the systemic venous circulation. These malformations are commonly associated with other congenital abnormalities, such as polysplenia and congenital heart defects. They can also lead to complications including liver dysfunction, hepatic encephalopathy, and the development of hepatocellular nodules ranging from focal nodular hyperplasia to hepatocellular carcinoma [1,2]

The following case illustrates the occurrence of a spectrum of hepatocellular nodules in the context of an Abernethy malformation in an 18 year old man. Molecular analysis of these lesions revealed different beta-catenin mutations, an occurrence associated with malignant transformation of hepatocellular adenoma [3]. This case highlights the potential of such lesions to undergo malignant transformation and the importance of having a high index of suspicion during their follow-up.

Case Report

An 18 year old man was under our care, with multiple nodular hepatic lesions secondary to type 1 Abernethy malformation. The diagnosis was made whilst investigating this young man for abnormal liver function tests but, in hindsight, suspicion regarding his vascular anatomy was raised as an infant when undergoing valvotomy for congenital pulmonary stenosis; the percutaneous procedure was converted to open due to difficulty proceeding through the inferior vena cava. There is no other significant medical history. The patient was not obese or diabetic.

He was investigated with magnetic resonance imaging (MRI) scan which demonstrated multiple hypervascular nodules in both lobes of the liver (fig 1 image a). The two dominant nodules inferiorly in segments 5/6 (fig 1 image b) measured 5 cms and 6 cms. There was absence of intrahepatic portal vein with the main portal vein draining into the retrohepatic vena cava (fig 1 image c). A further follow up MRI 3 months later demonstrated an increase in the size of the dominant segment 5 nodule from 6 cm to 8 cm (fig 1 image d).

This finding and deteriorating liver function precipitated the decision to perform an orthotopic liver transplant. The explanted liver weighed 1,330g, the portal vein was identified but its branches could not be followed through into the hepatic parenchyma. The background liver showed a range of changes affecting the small intrahepatic branches of the portal vein ranging from obliteration, attenuation of their profile, disarray or absence of the muscle wall. . Associated changes included ectasia of inlet venules and areas of sinusoidal dilatation, porto-septal fibrosis, parenchymal atrophy, hepatic plate disarray and in places nodular regenerative hyperplasia. Focal deposits of copper-binding protein were present at the limiting plate and there was a minimal degree of hepatocellular siderosis. Seven principal hepatocellular nodules were present; they had a tan cut surface and ranged in size from 10mm to 80mm. Multiple scattered nodules measuring up to 1mm were also present. Histologic examination revealed some of the nodules to have features of focal nodular

hyperplasia (FNH), others hepatocellular adenoma (HCA), and one of the largest, corresponding to the one which had increased in size, some atypical features and in particular focal loss of reticulin staining. This lesion also showed pseudogland formation as well as intralesional congestion, telangiectasia and peliosis in places along with some haemorrhage which may have contributed to the interval size change. There was no definite evidence of malignancy, however. The overall appearance, was short of overt hepatocellular carcinoma and the tumour was best classified as a well differentiated hepatocellular lesion [4] [fig 2]. All the lesions had a low proliferative rate and mitotic figures were not identified.

On immunohistochemistry, beta-catenin expression was confined to the membrane of lesional cells with no evident nuclear expression in any of the lesions. Glutamine synthetase expression was variable and ranged from map-like, to patchy and diffuse. One other lesion, with a diffuse glutamine expression pattern, also showed a perilesional rim of glutamine synthetase positive non-lesional hepatocytes. Fatty acid binding protein expression was preserved in all lesions tested. The serum amyloid A expression was focal in some of the lesions and confined to the lesional hepatocytes facing the supporting stroma or the peliotic areas, but was not sufficient to characterise these lesions as inflammatory adenomas. Immunohistochemistry for c-reactive protein did not show a significant overexpression in comparison with the background liver in most lesions. The HCA-like lesions showed a diffuse pattern of sinusoidal staining with CD34, whereas this staining was more patchy in the FNH-like areas. There was no staining for glypican-3 in any of the lesions tested.

Beta-catenin gene (*CTNNB1*) analysis was performed by PCR and sequencing of exons 2-4, 7 and 8 using DNA extracted from frozen tissue from six of the principal nodules (one extraction from each) and the background liver (two extractions). The background liver showed only wildtype sequence, whilst five of the nodules exhibited changes in exon 3: four a c.413T>C (p.Ser45Pro) change and one a c.414C>T (p.Ser45Phe) change. The final nodule carried a change in exon 7, c.1004A>T (p.Lys335Ile). No mutations in exon 8 were identified. The exon 3 variants occur at a casein kinase I phosphorylation site, which are known to be pathogenic and have previously been reported in a variety of tumour types, including HCC (<http://omim.org/entry/116806#0013>). Exons 7 and 8 of the beta-catenin gene have recently been reported to contain additional mutation hotspots for HCA, and potentially HCC, development. The presence of separate nodules, each with a different form of Beta-catenin protein (wild type, p.Ser45Pro, p.Ser45Phe, or p.Lys335Ile) on a background of wildtype liver suggests several independent genetic events have occurred in this liver and that a risk of neoplastic progression for some of these lesions exists independent of their histological appearance. Table 1 describes the histological features, immunohistochemical profile and beta-catenin mutation status of the lesions observed. No mutation in the telomerase reverse transcriptase (TERT) promoter mutation was identified.

Discussion

This case report is the first to demonstrate hepatocellular nodules with different beta-catenin mutations arising on a background of Abernethy malformation. Beta-catenin mutations are considered to be a risk factor for malignant transformation of HCA to HCC and it is therefore important to consider the possibility of such an event when monitoring people with Abernethy malformation.[3,5]

Abernethy malformations are congenital extrahepatic portosystemic shunts, which can be further classified using the anastomotic pattern between the portal and systemic veins. Type 1 exhibit complete absence of intrahepatic portal venous branches (end-to-side shunt) and can also be known as congenital absence of the portal vein. In type 2 there is preservation of intrahepatic venous supply but some portal flow is diverted through a side-to-side shunt into a systemic vein. [6]

Patients with type 1 malformations have a female predominance (74%) and typically present early in life with associated congenital abnormalities which can be intra-abdominal or cardiac.[1,6] Those with type 2 malformations are less likely to be affected by concomitant congenital defects and tend to present later in life with no sex preference.[1] The diagnosis of Abernethy malformation is often made when investigating infants and children for non-specific liver dysfunction and its sequelae, including hypoglycaemia, hyperammonaemia, hepatic encephalopathy, and portopulmonary hypertension.[1,6] Imaging studies are diagnostic but liver biopsies may be used to supplement these modalities as histology may reveal changes to small portal vein branches or associated secondary parenchymal changes which would be indicative of an underlying anomaly of the vascular supply.[6]

Approximately 50% of cases of Abernethy malformation are associated with hepatocellular nodules, ranging from FNH to HCC. A similar association has been described with other hepatic vascular disorders. [2,6] A recent retrospective multicentre survey of hepatic vascular disorders with associated well differentiated hepatocellular nodules highlighted the need to consider these lesions as potentially different from the conventional ones arising in association with oral contraceptives use. Such lesions are likely to have a different pathogenesis and natural history, and greater risk of malignant transformation.[2]

Beta-catenin is an adherens junction protein involved in the Wnt signalling pathway, it is encoded by the 16 exon *CTNNB1* gene at 3p22.1, (<http://omim.org/entry/116806#0013>). Disruption of beta-catenin phosphorylation prevents degradation of the molecule causing constitutive activation of various TCF/LEF transcription factors and is associated with several malignancies (<http://omim.org/entry/116806#0013>).[3] HCAs with beta-catenin mutations are considered to be at risk of malignant transformation when compared to other subtypes and have been identified in HCCs. [3,7] Indeed, the three beta-catenin variants found in these hepatocellular nodules, c.413T>C (Ser45Pro), c.414C>T (Ser45Phe) and c.1004A>T (Lys335Ile) have previously been identified in a series of HCAs and HCCs.[7,8]

There are two particular aspects that need to be considered in this case. The first one is the occurrence of different types of beta-catenin mutation in the hepatocellular nodules but not in the background liver. Whether nodule formation related to the underlying vascular anomaly precedes the onset of beta-catenin mutation or the mutation precedes nodule development remains uncertain. Systemic shunting of blood causes non-specific liver disturbance due to the unbalanced regional blood flow and arterialisatation. It is thought that this leads to relative hepatic ischaemia and compensatory increase in hepatic arterial flow, in addition to altered exposure of hepatocytes to hormones and growth factors.[6] These factors could lead to abnormal liver development, function and regeneration and could provide a stimulus to tumour development.[6]

The second aspect is that the beta-catenin mutations have occurred in nodules of different types including those resembling focal nodular hyperplasia histologically. Interestingly, transcriptome analysis of FNH has demonstrated activated hypophosphorylated beta-catenin protein accumulated in the absence of activating mutations.[9] The larger of the two lesions in this case report had an increase pattern of glutamine synthetase expression which deviated from the typical map-like one described by Bioulac-Sage et al.[10] and which could provide a clue to an underlying abnormality of the beta-catenin pathway. This supports the idea that hepatocellular lesions developing in the context of Abernethy malformation should be considered at risk of malignant progression independent of their size, histological and possibly radiological appearance, and that these patients should be followed up very closely. The multifocal nature of these lesions and genotypic heterogeneity indicate a field change, and emphasises the role of liver transplantation as a curative method in comparison with surgical resection targeted to more suspicious lesions.

Conclusion

This case report demonstrates that discrete beta-catenin mutations associated with malignant transformation can occur in hepatocellular nodules arising on a background of Abernethy malformation, independent of their size and histological appearance. The precise etiology is unclear, but may be common to other hepatic vascular disorders. It is therefore important to consider the possibility of malignant transformation of hepatocellular nodules when monitoring patients with Abernethy malformation, institute proper and close follow-up protocols and consider liver transplantation at a relatively early stage.

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Figure 1: Imaging of the liver

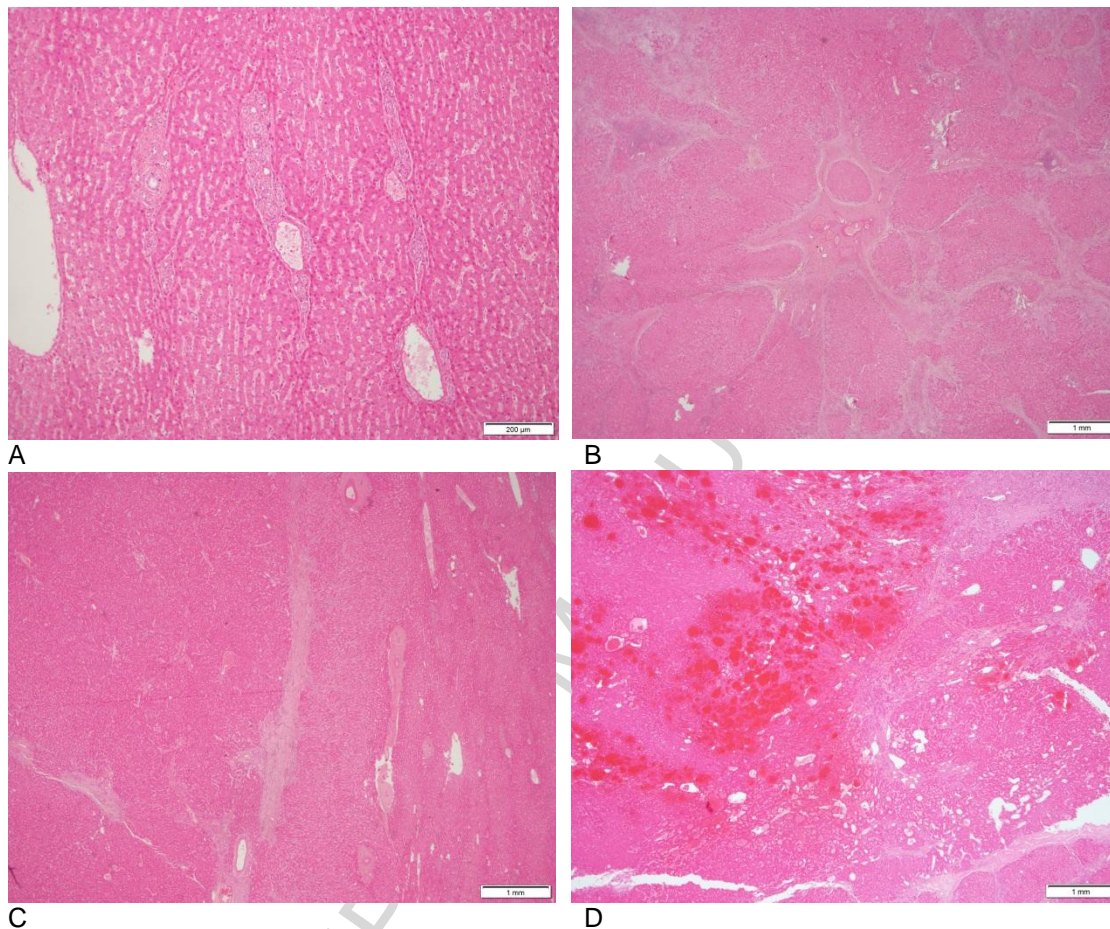
Figure 2: Photomicrographs representative of the background liver and nodular lesions

Table 1: *CTNNB1* exon 2-4, 7 and 8 mutational analysis

Figure 1: Imaging of the liver

Figure 1. MRI scan arterial phase images (A & B) demonstrating multiple hypervascular nodules in both lobes of the liver with a dominant nodule in segment 5 (arrow). Venous phase MR (C) depicting large porto-caval shunt (block arrow) and absence of intrahepatic portal venous branches. Follow up MR 3 months later (D) demonstrating an increase in the size of the dominant lesion in segment 5 (arrow).

Figure 2: Photomicrographs representative of the background liver and nodular lesions



A: Background liver showing changes affecting the small intrahepatic branches of the portal vein including from obliteration, attenuation of their profile, and disarray of their muscle coat. Associated changes seen include ectasia of inlet venules and areas of sinusoidal dilatation, porto-septal fibrosis, parenchymal atrophy, and hepatic plate disarray.

B: Focal nodular hyperplasia (tumour 2).

C: Hepatocellular adenoma (tumour 1).

D: Well differentiated hepatocellular neoplasm (tumour 6) exhibiting some atypical features and pseudogland formation as well as intralobular congestion, telangiectasia and peliosis with focal haemorrhage which may have contributed to the interval size change.

Table 1: Beta-catenin (*CTNNB1* exons 2-4, 7 and 8) histological mutational analysis

Tissue	Nodule size	Histologic Phenotype	Immunohistochemical Profile								Variant	Status
			Beta Cat	GS	SAA	CRP	FABP	CD34	Glypican-3	Reticulin		
Background liver		Changes affecting small intrahepatic branches of the portal vein	Membranous	Normal pattern	Normal pattern	Normal pattern	Preserved	Normal pattern	No staining	Normal	None detected	Normal only detected
Tumour 1	6 cm	Hepatocellular adenoma	Membranous, focal cytoplasmic. No nuclear staining	Diffuse	Focal	Similar to background liver	Preserved	Diffuse sinusoidal staining	No staining	Preserved	c.413T>C; p.(Ser45Pro)	Variant and normal detected
Tumour 2	1 cm	Focal nodular hyperplasia	Membranous. No nuclear staining	Patchy	Focal	Similar to background liver	Preserved	Patchy sinusoidal	No staining	Preserved	c.414C>T; p.(Ser45Phe)	Variant and normal detected
Tumour 3	1 cm	Focal nodular hyperplasia	Membranous. No nuclear staining	Map-like	Focal	Similar to background liver	Preserved	Patchy sinusoidal	No staining	Preserved	c.1004A>T; p.(Lys335Ile)	Variant and normal detected
Tumour 4	1.5 cm	Focal nodular hyperplasia	Membranous, focal cytoplasmic. No nuclear staining	Patchy	Focal	Similar to background liver	Preserved	Patchy sinusoidal, focally diffuse	No staining	Preserved	c.413T>C; p.(Ser45Pro)	Variant and normal detected
Tumour 5	2 cm	Hepatocellular adenoma	Membranous, focal cytoplasmic	Diffuse	Focal	Similar to background liver	Preserved	Patchy sinusoidal,	No staining	Preserved	c.413T>C; p.(Ser45Pro)	Variant and normal

			c. No nuclear staining					focally diffuse				detected
Tumour 6	8 cm	Well differentiated hepatocellular neoplasm*	Membranous. No nuclear staining	Patchy	Focal	Focally overexpressed	Preserved	Diffuse	No staining	Focal loss	c.413T>C; p.(Ser45Pro)	Variant and normal detected

Two DNA extractions on background liver were performed and one extraction each from the 6 tumours.

*Tumour 6 was radiologically suspicious for transformation into hepatocellular carcinoma in part due to relatively rapid enlargement. Histologically, this lesion had some atypical features but fell short of an overt hepatocellular classification and is best classified as well differentiated hepatocellular neoplasm. The interval enlargement was explained by intralesional haemorrhage.



Figure 1